

CLAIMS

1) A method for determining the level of free protein S in a biological fluid, such as blood, plasma or serum, said method comprising the steps

(a) bringing a ligand, that binds specifically to free protein S, into contact with said

5 biological fluid to form a reaction mixture comprising at least a liquid phase;

(b) maintaining said reaction mixture for a time period sufficient for said ligand to bind to free protein S in said fluid and thereby to form a protein S/ligand complex; and

(c) determining the level of the complex formed in step (b) and thereby the level of free protein S in said fluid,

10 in which method the ligand of step (a) is comprised of (i) at least part of the C4b-binding protein (C4BP), said at least part of C4BP comprising at least part of the protein S binding site of C4BP; (ii) a compound comprising an amino acid residue sequence homologous or analogous to the protein S binding site of C4BP; or (iii) a compound having essentially the same protein S binding specificity as C4BP and comprising an amino acid residue sequence
15 that binds specifically to the binding site for C4BP in protein S.

2) The method of claim 1, wherein said ligand of step (a) is operatively linked to a solid carrier, the reaction mixture formed in step (a) being comprised of a liquid phase and a solid phase and the complex formed in step (b) being comprised in the solid phase linked to the carrier.

20 3) The method of claim 1, wherein the reaction mixture formed in step (a) is comprised of one phase, which is a liquid phase.

4) The method of any of claims 1, 2 and 3, wherein an indicating means is added to the mixture of step (a), said indicating means being added separately or in a form wherein it is operatively linked to or incorporated in the ligand and said indicating means
25 being capable of producing, directly or indirectly, a detectable signal at the formation of the complex in step (b), and wherein the complex formed in step (b) comprises the indicating means and the determination in step (c) comprises measuring the amount of indicating means in said complex.

5) The method of claim 2, wherein the determination in step (c) comprises
30 contacting the carrier-bound protein S-containing complex formed in step (b) with an antibody specific for protein S to form an immunoreaction mixture having a liquid and a solid phase and for a time period sufficient for said antibody to immunoreact with said

complex to form an immunoreaction product linked to the solid phase; and determining the amount of the said antibody present in the said immunoreaction product, and thereby the amount of said protein S-containing complex formed in step (b).

6) The method of claim 5, wherein the antibody specific for protein S is an
 5 antibody having high affinity for protein S, that is designated HPS54 and binds to a site in protein S that is distinct from the binding site for C4BP in protein S, said antibody optionally carrying an indicator means enabling determination of the amount thereof bound to protein S in the protein S/ligand complex.

7) The method of any of claims 1-6, wherein the ligand used in step (a) is compri-
 10 sed of C4BP.

8) The method of any of claims 1-6, wherein the ligand used in step (a) is compri-
 sed of at least one fragment of C4BP, said fragment comprising the protein S binding site of C4BP; a hybrid molecule comprised of said C4BP fragments; or a compound comprising an amino acid sequence homologous or analogous to the protein S binding site of C4BP.

9) The method of any of claim 1-6, wherein the ligand used in step (a) is compri-
 15 sed of a polyclonal or monoclonal antibody, that immunoreacts with the C4BP binding site in protein S; or a fragment thereof.

10) The method of claim 9, wherein said antibody, or a fragment thereof, immuno-
 reacts with an amino acid sequence comprising the amino acid residues numbered 447-460
 20 in mature protein S and represented by the formula SGIAQFHIDY NNVS, or an amino acid sequence homologous or analogous therewith.

11) The method of any preceding claim, wherein steps (a) and (b) are performed in the presence of Ca^{++} ions.

12) An antibody specific for protein S, which antibody is polyclonal or, suitably,
 25 monoclonal and is specific for at least part of the binding site for C4BP in protein S comprising a sequence of the amino acid residues numbers 447-460 in mature protein S represented by the formula SGIAQFHIDY NNVS; and which antibody immunoreacts with a fragment derived from protein S or a protein S related polypeptide comprising said sequence, or an amino acid residue sequence homologous or analogous therewith; and
 30 further immunoreacts with free protein S but substantially not with protein S bound to C4BP.

13) The antibody of claim 12, wherein said antibody is monoclonal and immuno-reacts with a protein S related polypeptide comprising the sequence of amino acid residues numbers 439-460, 447-468 or 435-468 of mature protein S represented by the formulas EKGSYYPGSG IAQFHIDYNN VS,

5 SGIAQFHIDY NNVSSAEGWH VN and
LVTVEKGSYYPGSGIAQFHI DYNNVSSAEG WHVN, resp., or an amino acid residue sequence homologous or analogous therewith.

14) Diagnostic test system, suitably in kit form, for assaying free protein S, said system comprising as separately packaged reagents or as a combination of at least two
10 reagents, the ligand of claim 1 and at least one further reagent required to perform the assay.

15) The system of claim 14 comprising the ligand operatively linked to a solid carrier, suitably a microtiter plate.

16) The system of claim 14 or 15, which further comprises an indicating means.

15 17) A polypeptide related to protein S, which polypeptide comprises an amino acid residue sequence containing the amino acid residues no 447-460 of mature protein S represented by the formula SGIAQFHIDY NNVS and which polypeptide binds to the binding site for protein S in C4BP and thereby inhibits the binding of protein S to C4BP.

18) The polypeptide of claim 17, which polypeptide has immunogenic and/or
20 antigenic properties and can be used to produce the antibody of claim 11.

19) The polypeptide of claim 17 and 18, which polypeptide comprises an amino acid residue sequence corresponding to residues no 439-460, 447-468 or 435-468 of mature protein S and represented by the formulas

EKGSYYPGSG IAQFHIDYNN VS,

25 SGIAQFHIDY NNVSSAEGWH VN and
LVTVEKGSYYPGSGIAQFHIDYNNVSSAEGWHVN, resp., or an amino acid residue sequence homologous or analogous therewith.
